

Morphological Variation of the Corona Radiata in *Oesophagostomum dentatum*, *O. quadrispinulatum*, and *O. radiatum* (Nematoda: Strongyloidea)

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ABSTRACT: The anterior end of 76 adult and of several juvenile *Oesophagostomum dentatum*, of 75 adult *O. quadrispinulatum*, and of 70 adult *O. radiatum* (Strongyloidea, Nematoda) was investigated by scanning electron and light microscopy. Both an external and an internal ring of buccal leaves (corona radiata externa and interna) are present in *O. dentatum* and *O. quadrispinulatum*, whereas a single ring of buccal leaves occurs in *O. radiatum*. Remnants of external buccal leaves indicate that the single ring of leaves found in the latter species is homologous to the corona radiata interna of *O. dentatum* and *O. quadrispinulatum*. The number of buccal leaves of the corona radiata varies remarkably in adults of all 3 species. There are 9–12 external leaves in *O. dentatum*, 9–11 external leaves in *O. quadrispinulatum*, and 30–40 internal leaves in *O. radiatum*. Nine leaves are most common in both *O. dentatum* and *O. quadrispinulatum*, but the former species shows a higher frequency of individuals with more than 9 leaves. In *O. radiatum*, buccal leaves usually occur in even numbers and very rarely in odd numbers. Small, regularly arranged protuberances outside the ring of buccal leaves may indicate reduced leaves of the corona radiata externa. Juveniles of *O. dentatum* do not possess buccal leaves, but a thin cuticular velum in the fourth stage and neither a corona nor a velum in the second and first stage.

KEY WORDS: *Oesophagostomum dentatum*, *Oesophagostomum quadrispinulatum*, *Oesophagostomum radiatum*, corona radiata, polymorphism.

Species of *Oesophagostomum* parasitize the alimentary tract of livestock and humans around the world (Murrell, 1986; Skryabin et al., 1992; Blotkamp et al., 1993; Roepstorff and Nansen, 1994). The anatomy of members of this taxon has been described by light microscopy (Goodey, 1924a; Chitwood, 1931; Blotkamp et al., 1993). Few observations by scanning electron microscopy (SEM) are available (Gibbons, 1986; Stewart and Gasbarre, 1989), and only 2 studies report on transmission electron microscopy (TEM) findings (Neuhaus et al., 1997a, b).

At its mouth opening, a member of the genus *Oesophagostomum* typically possesses a corona radiata externa and interna composed of a variable number of buccal leaves that are of taxonomic value (Chabaud and Durette-Desset,

1974; Lichtenfels, 1980; Skryabin et al., 1992; Hartwich, 1994). Polymorphism has been reported for the number of buccal leaves of *O. asperum* Railliet and Henry, 1913, *O. bifurcum* (Creplin, 1849), *O. brevicaudum* Schwartz and Alicata, 1930, *O. columbianum* (Curtice, 1890), *O. dentatum* (Rudolphi, 1803), *O. radiatum* (Rudolphi, 1803), *O. sikae* Cameron and Parnell, 1933, and *O. venulosum* (Rudolphi, 1809) (cf. Rudolphi, 1803; Goodey, 1924b; Schwartz and Alicata, 1930; Blotkamp et al., 1993; Hartwich, 1994). However, no information is available about how commonly a given number of leaves occurs within a population. The object of this study is to provide data about the frequency distribution of the number of external buccal leaves in *O. dentatum* and *O. quadrispinulatum* (Marcone, 1901) and of internal leaves in *O. radiatum*.

Materials and Methods

Adult specimens and fourth-stage juveniles of *O. dentatum* and adult specimens of *O. quadrispinula-*

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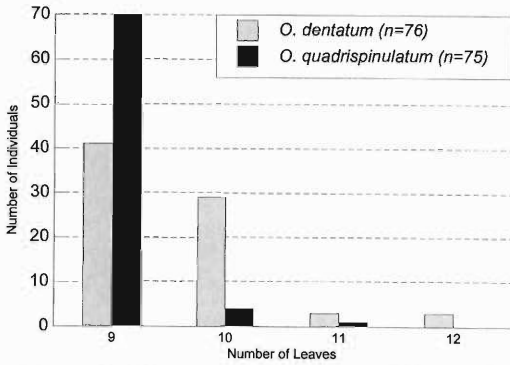


Figure 1. Frequency distribution of the number of leaves in the corona radiata externa of *Oesophagostomum dentatum* ($n = 76$) and *O. quadrispinulatum* ($n = 75$).

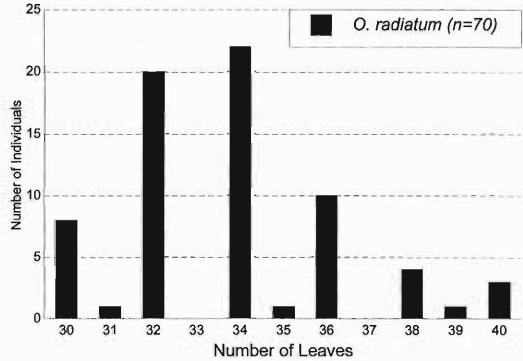


Figure 2. Frequency distribution of the number of leaves in the corona radiata interna of *Oesophagostomum radiatum* ($n = 70$).

tum were obtained from 2 experimentally infected pigs. Adult *O. radiatum* were collected from an experimentally infected calf. All specimens for SEM were taken at random from the nematodes sampled and were further treated for SEM and light microscopy as described by Neuhaus et al. (1997a). A subsample of about 100 nematodes from each of the infected pigs was studied by light microscopy to ensure that only a single species of *Oesophagostomum* occurred in each pig. The following morphological characters also used by Haupt (1966), Taffs (1967), Kendall et al. (1977), Poelvoorde (1978), and Hartwich (1994) were checked to discriminate *O. dentatum* from *O. quadrispinulatum*: shape of buccal capsule and of pharynx, length of spicules, and distances between tail and anus as well as between anus and vulva. Specimens of *O. radiatum* were identified according to the key by Hartwich (1994).

Eggs of *O. dentatum* were cultivated through to the third juvenile stage on agar plates as described in Neuhaus et al. (1997a). Approximately 10 specimens of each juvenile stage were studied by light microscopy and SEM, respectively.

Results

Adult specimens of *Oesophagostomum dentatum* and *O. quadrispinulatum* possess a characteristic lobed or bilobed head region (Figs. 3–5), whereas *O. radiatum* appears to show a trilobed head region (Figs. 14–16); the anteriormost lobe consists of the mouth collar, which is significantly enlarged in this species. In fixed material, alae of *O. radiatum* are very prominent in comparison with *O. dentatum* and *O. quadrispinulatum* (Figs. 3, 4, 15). The number of buccal leaves of the corona radiata at the anterior mouth opening of adult *Oesophagostomum* is difficult to observe with the light microscope (Figs. 5, 14) but can be readily seen

in frontal view of clean specimens using SEM (Figs. 6–8, 13, 18–22). The number of leaves of the corona radiata interna in *O. dentatum* and *O. quadrispinulatum* is extrapolated from the observed number of internal leaves at the base of each external leaf.

Adult *Oesophagostomum dentatum* ($n = 76$)

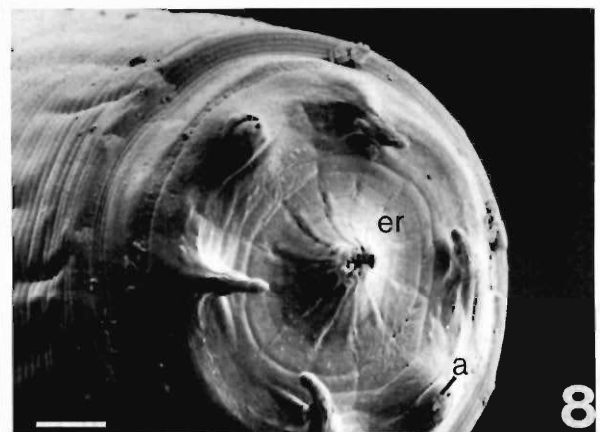
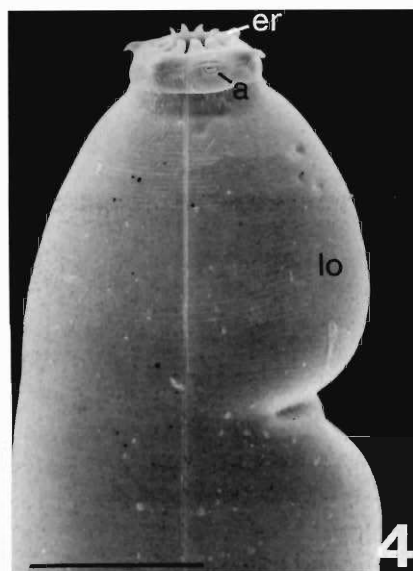
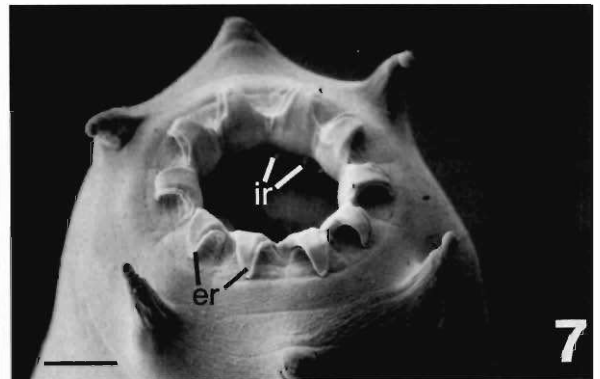
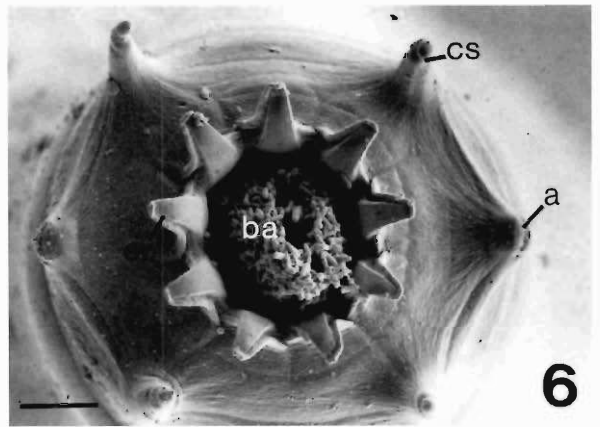
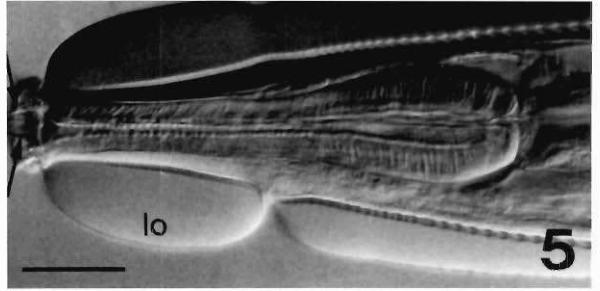
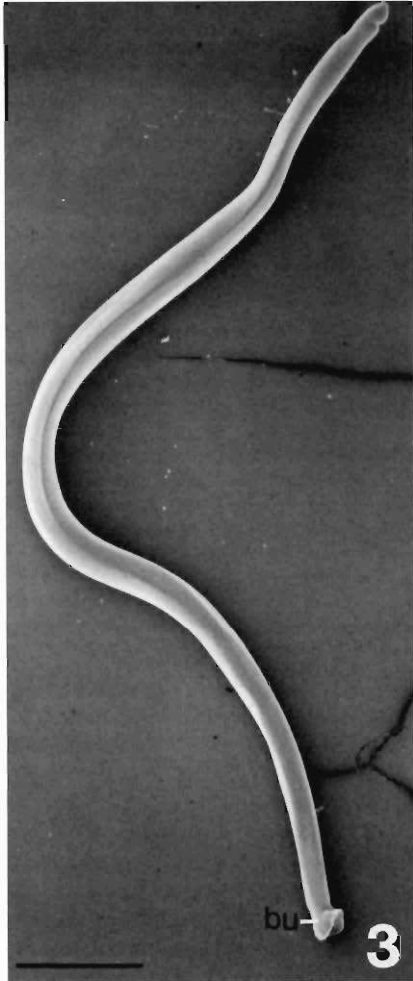
A variable number of 9–12 elements of the corona radiata externa (external buccal leaves) surrounds the mouth opening of adult *O. dentatum*. Numbers of 9 or 10 leaves (Figs. 6, 7) are most common (Fig. 1). The triangular leaves of the corona radiata may close the mouth opening almost completely (Fig. 8). At the base of each external leaf, 2 considerably smaller, triangular, tooth-like internal leaves are found (elements of the corona radiata interna) (Fig. 7; Fig. 12 for *O. quadrispinulatum*). Bacteria often occur in the buccal lumen (Fig. 6).

Juvenile *O. dentatum*

A corona radiata is missing completely in all juvenile stages (Figs. 9–11). A thin cuticular velum covers the mouth opening partly in the fourth stage (Fig. 11) but is missing in the first and second stages (Figs. 9, 10). The anterior end of the free-living third stage was not available for SEM studies, because this stage is ensheathed by the cuticle of the second juvenile stage.

Adult *Oesophagostomum quadrispinulatum* ($n = 75$)

Adult *O. quadrispinulatum* exhibit 9–11 triangular buccal leaves in the corona radiata ex-



terna. Almost all specimens possess 9 leaves (Fig. 13), whereas few individuals show 10 or 11 leaves (Fig. 1). The corona radiata interna is arranged as in *O. dentatum* (Fig. 12). Shape and morphology of the buccal leaves are similar to *O. dentatum*.

Adult *Oesophagostomum radiatum* ($n = 70$)

Adult *O. radiatum* possess a single ring of buccal leaves (Figs. 18–22), which agree in shape, morphology, and size with the leaves of the corona radiata interna of *O. dentatum* and *O. quadrispinulatum*. The leaves are usually arranged in pairs (Figs. 17–19, 22) but appear very seldom in odd numbers (Figs. 2, 20, 21). They are not able to close the mouth opening even partly (Figs. 16, 19, 21). Occasionally, individual leaves are smaller than the neighboring leaves (Figs. 19–21). The number of buccal elements varies between 30 and 40, with 32 and 34 leaves being most common (Fig. 2).

Outside the ring of buccal leaves, a ring of small, regularly arranged protuberances is found (Figs. 17–19, 22). These projections are always located between neighboring pairs of buccal leaves.

Discussion

At their anterior end, many species of *Oesophagostomum* possess both a corona radiata externa and interna composed of several to many buccal leaves. At the base of each element of the corona externa, 2 leaves of the corona interna appear (e.g. Chabaud and Durette-Desset, 1974; Lichtenfels, 1980; this paper). Our findings reveal, in agreement with earlier observations (Goodey, 1924a, b; Stewart and Gasbarre, 1989), 2 coronae radiatae for both *O. dentatum* and *O. quadrispinulatum* and a single ring of buccal leaves for *O. radiatum*. Shape and size of the buccal leaves of the latter species has lead other investigators to the conclusions that the buccal leaves of *O. radiatum* rep-

resent elements of the corona radiata interna, but a corona externa is missing (e.g. Goodey, 1924b; Travassos and Vogelsang, 1932). The ring of regularly arranged, small protuberances outside the corona radiata of *O. radiatum* confirms the aforementioned assumption; the protuberances are interpreted as remnants of the corona radiata externa.

The arrangement and number of the elements of the coronae radiatae differ considerably in the taxon *Oesophagostomum*. The following patterns have been found: (1) 6–8 external leaves, no corona radiata interna (e.g., *O. oldi* Goodey, 1924, *O. mwanzae* Daubney, 1924, *O. eurycephalum* Goodey, 1924, *O. simpsoni* Goodey, 1924 [cf. Goodey, 1924c]); (2) no corona radiata externa, 38–45 leaves of the corona interna (e.g., *O. radiatum* [but compare our results], *O. curvatum* Maplestone, 1931, *O. sikae* Cameron and Parnell, 1933, and *O. traguli* (Chandler, 1931) [cf. Goodey, 1924b; Chabaud and Durette-Dussset, 1974]); (3) 30–40 external leaves, 60–80 internal leaves (e.g., *O. pachycephalum* Molin, 1861, *O. stephanostomum* Stossich, 1904, *O. ventri* Thornton, 1924 [cf. Glen and Brooks, 1985]); and (4) 9–24 external leaves and 18–48 internal leaves in the remaining species of *Oesophagostomum* (cf. Chabaud and Durette-Desset, 1974; Lichtenfels, 1980). From these light microscopical investigations, it remains open whether internal leaves have not been overlooked in the first group of taxa because of their small size. Remnants of external leaves are almost invisible in the second group of *Oesophagostomum* species as has been revealed by our observations of *O. radiatum*. We therefore suppose that the corona radiata externa has been reduced not only in *O. radiatum* but also in *O. curvatum*, *O. sikae*, and *O. traguli*.

It has been assumed that 6–8 external leaves represent the original condition for *Oesophagostomum* retained only in few species, where-

Figures 3–8. Adult *Oesophagostomum dentatum*. 3, 4. SEM of male worm in lateral view. 4. Higher magnification of lobed head region with prominent mouth collar. 5. Differential interference contrast microphoto of anterior end in lateral view. Arrows mark leaves of corona radiata externa. 6–8. SEM frontal view of specimen with 9 external leaves and bacteria in buccal cavity (6), with 10 external leaves (7), or with 12 external leaves in closed position (8). Abbreviations: a, amphid; ba, bacteria; bu, bursa; cs, cephalic sensillum; er, leaf of corona radiata externa; ir, leaf of corona radiata interna; lo, lobe of head region. Scale bar in 3 -1 mm, in 4 and 5 -100 μ m, and in 6–8-10 μ m.

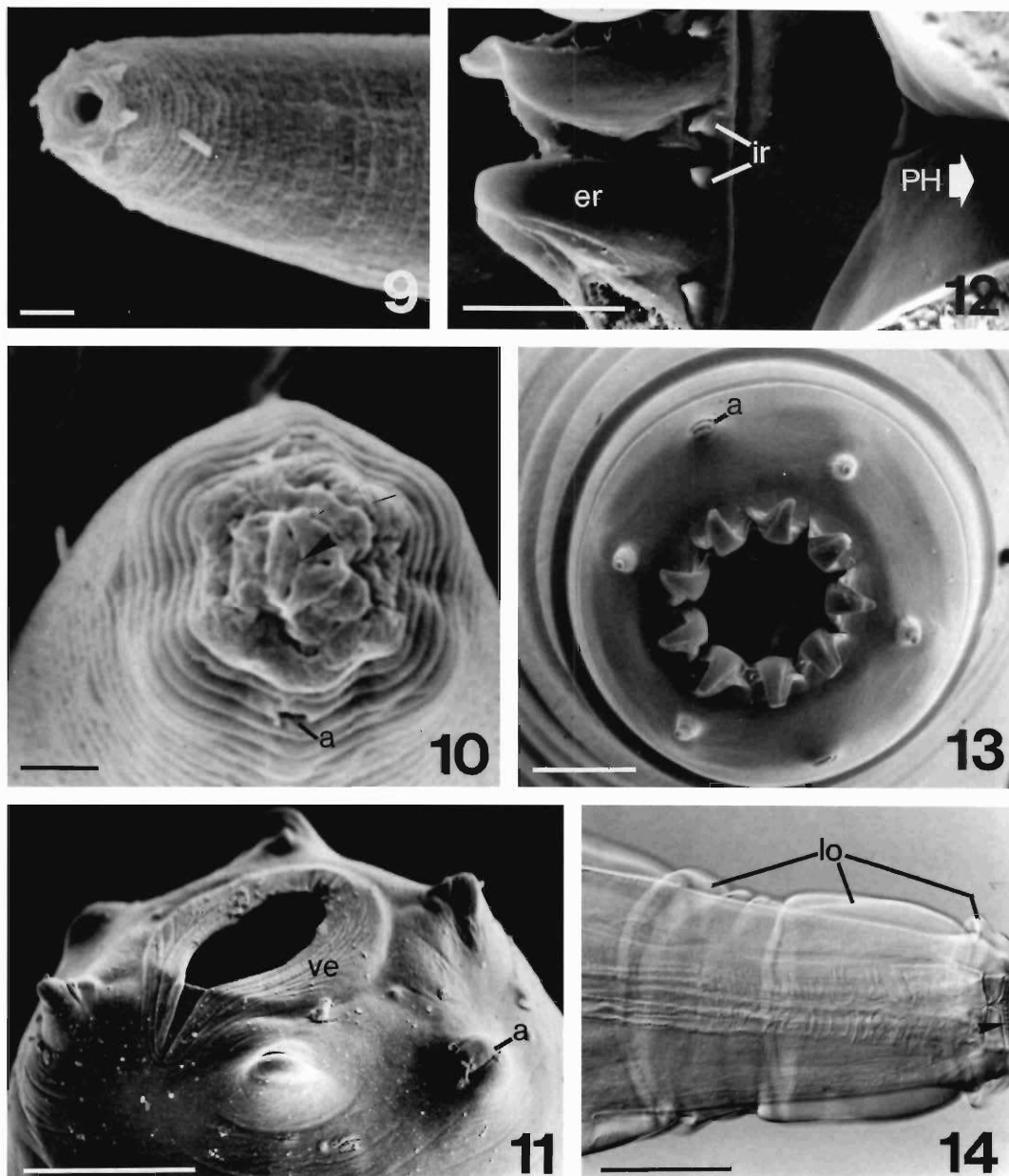


Figure 9–14. *Oesophagostomum* species. 9–11. Juvenile *O. dentatum*. 9. SEM of juvenile stage 2 with open mouth opening. 10. SEM of juvenile stage 3 showing cuticle of previous stage with collapsed mouth opening. 11. SEM of juvenile stage 4 with velum partly covering mouth opening. 12, 13. Adult *O. quadrispinulatum*. 12. SEM view into opened buccal cavity with leaves of corona radiata externa and interna. Arrow points toward pharynx. 13. SEM frontal view. 14. Differential interference contrast photo of anterior end of adult *O. radiatum* with trilobed head region. Arrowhead marks leaves of corona radiata. Abbreviations: a, amphid; er, leaf of corona radiata externa; ir, leaf of corona radiata interna; lo, lobe of head region; PH, pharynx; ve, velum. Scale bar in 9 and 10–2 μm , in 11 and 12–10 μm , in 13–20 μm , and in 14–100 μm .

Table 1. Polymorphism in the corona radiata externa and interna of various species of *Oesophagostomum*.

| Species | Number of external leaves | Number of internal leaves | Reference |
|----------------------------|---------------------------|---------------------------|---|
| <i>O. asperum</i> | 10–14 | 32–40 | Hartwich, 1994* |
| <i>O. bifurcum</i> | 14–16 | 28–32 | Blotkamp et al., 1993 |
| <i>O. brevicaudum</i> | 14–16 | 28–32 | Schwartz and Alicata, 1930 |
| <i>O. columbianum</i> | 20–26 | 40–52 | Hartwich, 1994* |
| <i>O. dentatum</i> | 10–12 | †? | Rudolphi, 1803 |
| | 9 | 18 | Goodey, 1924a; Hartwich, 1994* |
| | 9–12 | 18–24 | This paper |
| <i>O. quadrispinulatum</i> | 9 | 18 | Stewart and Gasbarre, 1989; Hartwich, 1994* |
| | 9–11 | 18–22 | This paper |
| <i>O. radiatum</i> | No corona | 32–40 | Hartwich, 1994* |
| | Reduced corona | 30–40 | This paper |
| <i>O. sikae</i> | No corona | 36–38 | Hartwich, 1994 |
| <i>O. venulosum</i> | 16–20 | 32–40 | Hartwich, 1994* |

* After different authors, maximal variation summarized by Hartwich (1994).

† ? = not mentioned.

as more than 8 external leaves (i.e., 9–24 leaves) are apomorphic (Chabaud and Durette-Desset, 1974; Glen and Brooks, 1985). The phylogenetic hypothesis on the evolution of the taxon *Oesophagostomum* presented by Glen and Brooks (1985, Fig. 3) suggests that the combination 30–40 external leaves and 60–80 internal leaves has developed from a condition with 9–24 external and 18–48 internal leaves and represents an autapomorphic character of *O. pachycephalum* + *O. stephanostomum* + *O. ventri*.

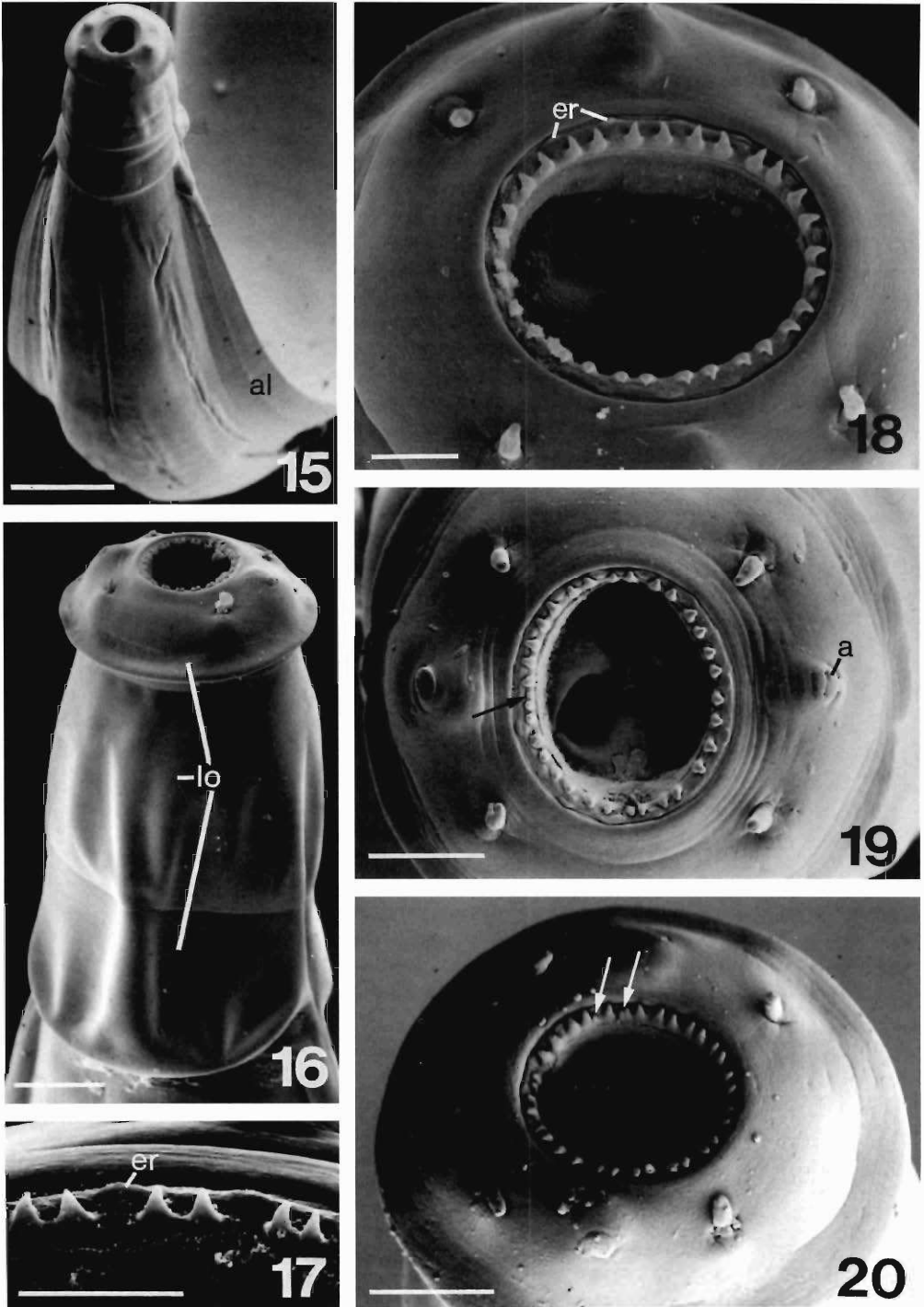
Previous taxonomic or morphological investigations usually mention the number of buccal leaves for different species of *Oesophagostomum* but have only occasionally checked a larger number of specimens for polymorphism (Table 1). Data about the frequency of a given number of leaves are missing entirely. In our material, the frequency distribution of the number of buccal leaves differs considerably between *O. dentatum* and *O. quadrispinulatum*, the latter species expressing by far less variation in the number of leaves. The reason for such differences is unknown, and there is no apparent functional necessity for varying the number of buccal leaves. Our observations and a brief literature review (Table 1) suggest that polymorphism is a common character in the corona radiata of species of *Oesophagostomum*. We assume that such a polymorphism reflects the genetic potential of the species. But, the extent to which polymorphism is expressed in *O.*

dentatum and *O. quadrispinulatum* (i.e., the frequency with which a certain number of leaves occurs) may be either species-specific or may depend on environmental influences during the ontogeny. In the latter case, the unfolding of the nematode morphotype may be less adversely influenced and hence the variability less pronounced under more optimal developmental conditions in the gut environment. Future investigations should be aware of polymorphism in the corona radiata of *Oesophagostomum* and, when appropriate, examine a larger number of individuals.

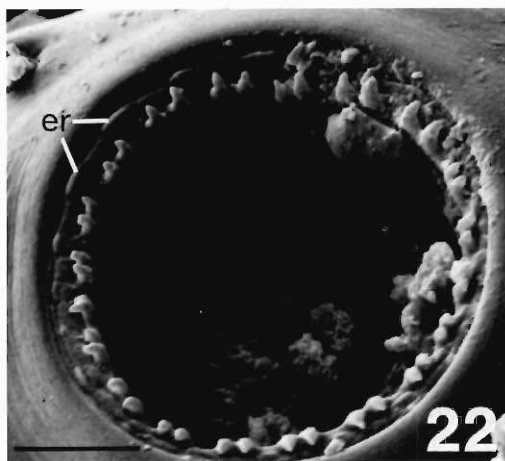
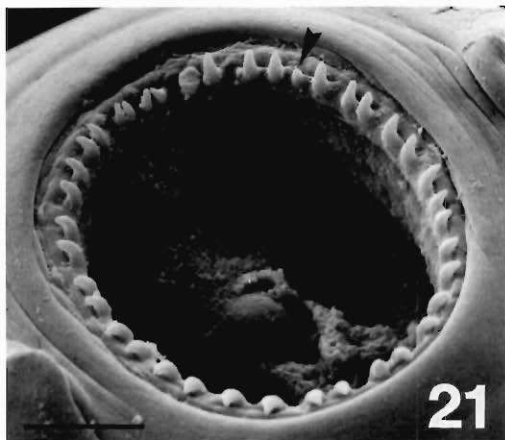
Polymorphism in the corona radiata of strongylid nematodes has also been reported for 18 species of the Cyathostominae (Braide and Georgi, 1974). A limited number of specimens (up to 18) per species was studied. Variation was either little or exceptionally large in few species but moderate in most species. No reasons for polymorphism were specified by Braide and Georgi (1974).

Acknowledgments

We appreciate the steady and encouraging interest and discussions with Professors Peter Nansen and Flemming Frandsen. The technical assistance by Bodil W. Jørgensen and Leif S. Jensen is gratefully acknowledged. We are indebted to the Danish National Research Foundation for financial support to the Danish Centre for Experimental Parasitology.



Figures 15–20. SEM of *Oesophagostomum radiatum*. 15. Anterior end with trilobed head region and prominent lateral alae in trunk region. 16. Trilobed head region at higher magnification. 17. Arrangement of leaves of corona radiata in pairs. 18–20. Frontal view of specimen with 30 leaves (18), 32 leaves (19), or 35 leaves (20). Arrows in 19 and 20 mark smaller leaves. Abbreviations: a, amphid; al, lateral ala; er,



Figures 21, 22. SEM frontal view of *Oesophagostomum radiatum* with 39 leaves (21) or 40 leaves (22). Arrowhead in 21 marks small leaf. Abbreviation: er, remnant of leaf of corona radiata externa. Scale bar in 21 and 22-10 μ m.

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remnant of leaf of corona radiata externa; lo, lobe of head region. Scale bar in 15–100 μ m, in 16, 19, and 20–20 μ m, and in 17 and 18–10 μ m.

- bellling with wheat germ agglutinin-gold conjugate indicating chitin in the pharyngeal cuticle of *Oesophagostomum dentatum* (Strongylida, Nematoda). Acta Zoologica. (In press.)
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